Enabling Biocatalysis by High Throughput Protein Engineering Using Droplet Microfluidics Coupled to Mass Spectrometry

(Supporting Information)

Xue W. Diefenbach[†‖], Iman Farasat[†], Erik D. Guetschow[†], Christopher J. Welch[†§], Robert T. Kennedy[‡], Shuwen Sun*[†], Jeffrey C. Moore*[†]

[†] Merck Research Laboratory, Merck & Co., Inc., 126 E Lincoln Ave., Rahway, NJ, 07065, USA

[‡] Department of Chemistry, University of Michigan, 930 N University Ave., Ann Arbor, MI, 48109, USA

[‖] 3579 NW Paisley Ct., Beaverton, OR, 97006, USA

[§] Welch Innovation, LLC., Cranbury, NJ, 08512, USA

*Corresponding authors

Tel: (+1) 732-594-6508

E-mail for Sun, S.: shuwen.sun@merck.com

E-mail for Moore, J.: jeffrey_moore@merck.com

Supporting Information

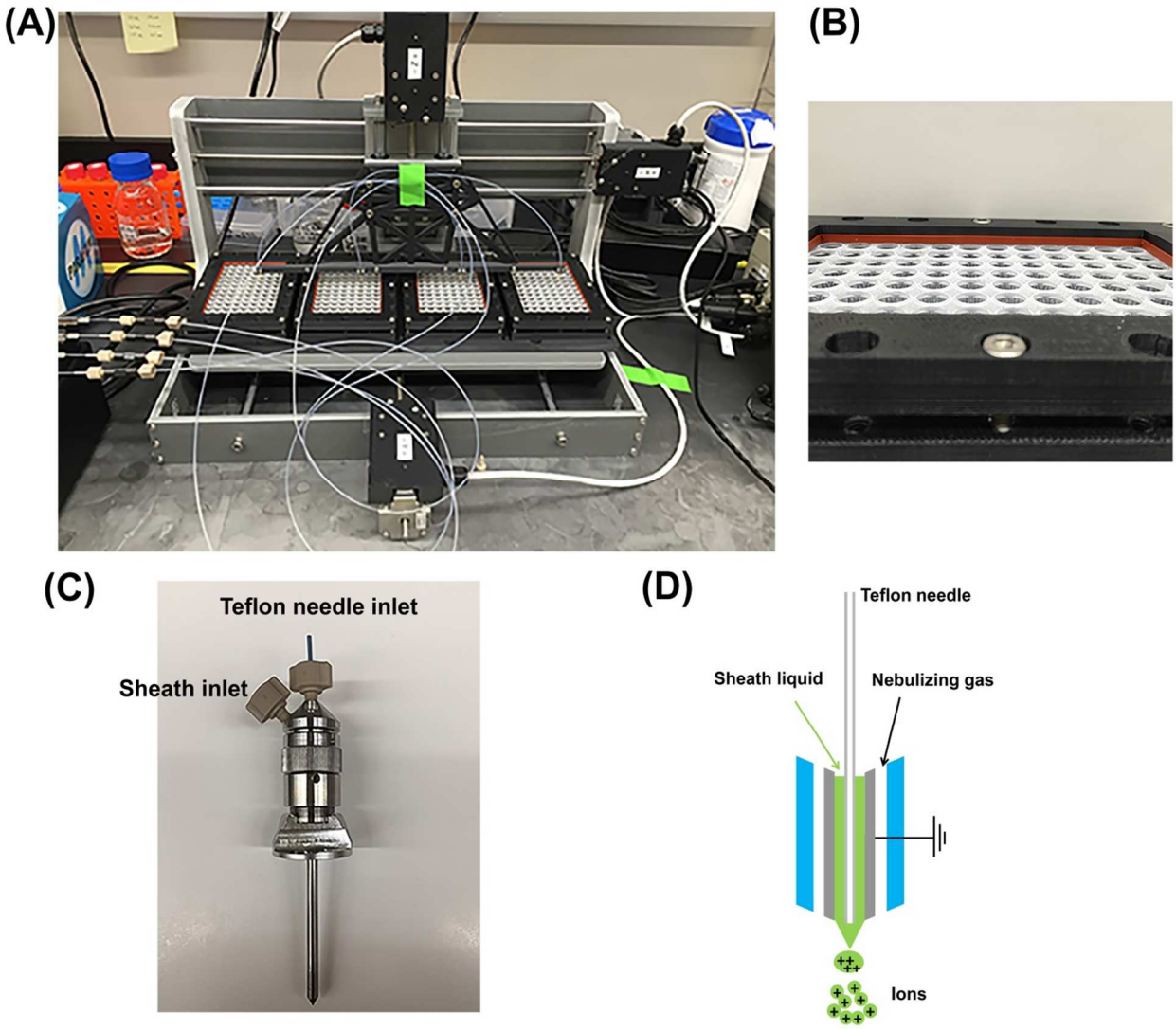
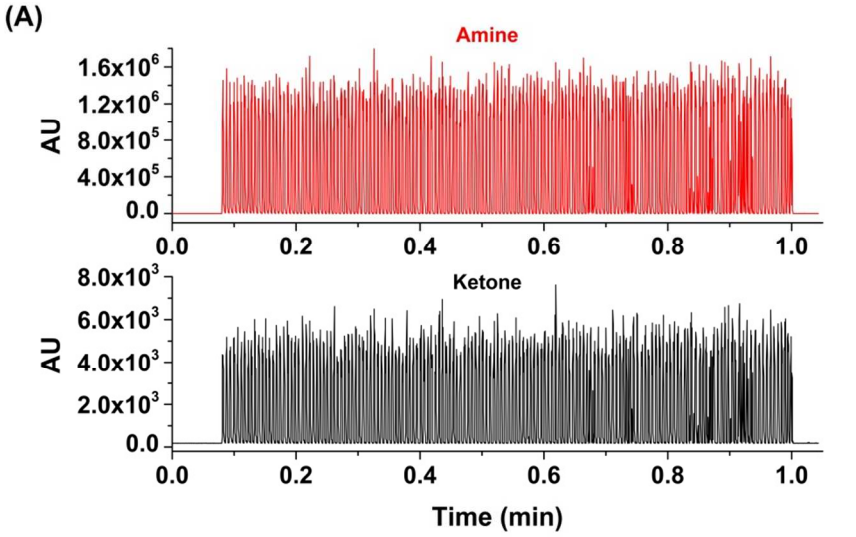
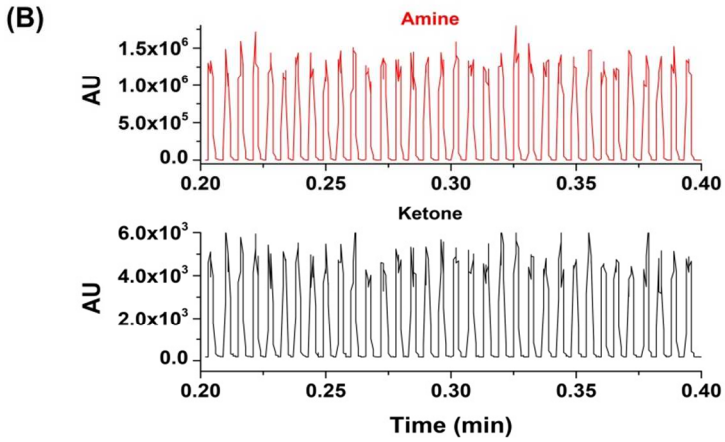
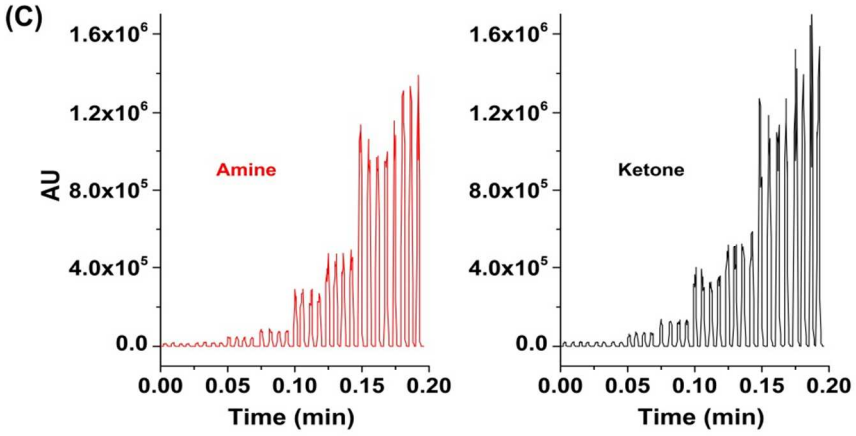


Figure S1. Droplet generation system (A) Setup: An XYZ positioner to control the movement of the droplet carrier Teflon tubing. The capacity is 4 multi-well plates. (B) Plate holder: a silicone gasket is secured on top of the outer edges of the 96 well-plate by sandwiching them between two 3D-printed holders. (C) Agilent CE-MS ESI source: Teflon ESI needle is inserted from the top inlet and the sheath flow is introduced from the side inlet. When no sheath flow applied, the inlet is plugged. (D) Scheme of the inside of the CE-MS source: a Teflon needle is inserted to the tip of the sprayer. Sheath liquid is delivered between the Teflon needle and the grounded stainless steel needle housing, mixing with the sample droplet at the tip of the source.


(A)
Amine
AU
1.6x10^6
1.2x10^6
8.0x10^5
4.0x10^5
0.0
0.0
0.2
0.4
0.6
0.8
1.0
Ketone
AU
8.0x10^3
6.0x10^3
4.0x10^3
2.0x10^3
0.0
Time (min)


(B)
Amine
AU
1.5x10^6
1.0x10^6
5.0x10^5
0.0
0.20
0.25
0.30
0.35
0.40
Ketone
6.0x10^3
4.0x10^3
2.0x10^3
Time (min)


(C)
AU
Amine
Ketone
1.6x10^6
1.2x10^6
8.0x10^5
4.0x10^5
0.0
0.00
0.05
0.10
0.15
0.20
Time (min)

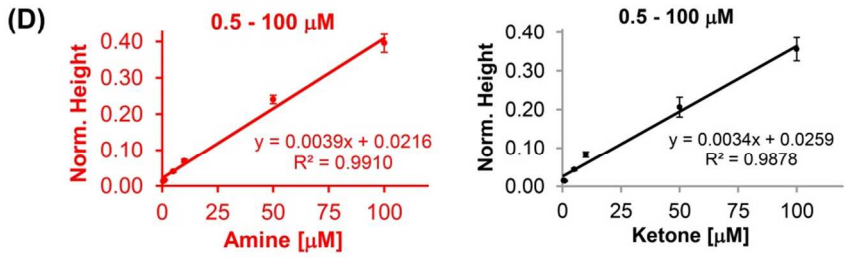

(D)
0.5 - 100 μM
Norm. Height
y = 0.0039x + 0.0216
R² = 0.9910
Amine [μM]
0.5 - 100 μM
y = 0.0034x + 0.0259
R² = 0.9878
Ketone [μM]

Figure S2. Signal stability and linearity using Teflon ESI needle: (A) Droplet traces of two SIM signals selected for pyridinyl amine and ketone. 200 droplets were continuously sprayed to the MS. Sample: 1 mM standard pyridinyl amine with trace amount of ketone. Analysis rate was 3.6 Hz. (B) Zoom-in droplet trace of 0.2-0.4 min from A. (C) A series of 8 concentrations of the standard pyridinyl amine and ketone were sampled into quadruplicated droplets and analyzed by MS. Concentrations were: 0.5 μM, 1 μM, 5 μM, 10 μM, 50 μM, 100 μM, 500 μM, 1000 μM. (D) Calibration curves plotted for amine and ketone based on C. Signals remain linear between 1 and 100 μM.

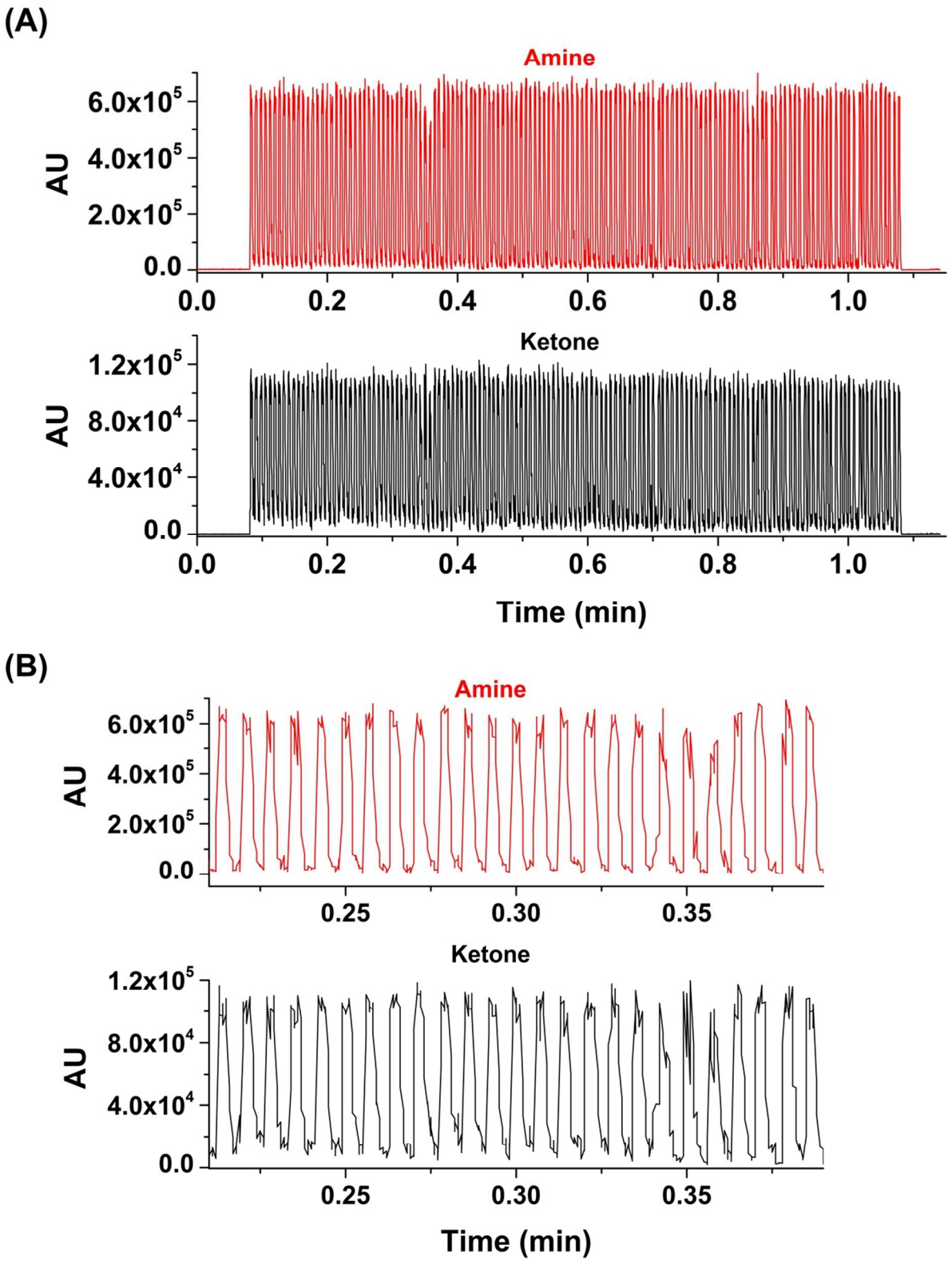


Figure S3. Evaluation of the impact of new oil-surfactant carrier fluid on MS signal: SIM signals of pyridinyl amine and ketone to be used in ivTT test. (A) 200 droplets from the same sample

were generated using the carrier fluid, HFE 7500 with 2% fluorosurfactant 008, which were then continuously sprayed in to the MS through a 120/250 Teflon needle. Droplet size: 50 nL. Droplet infusing rate: 15 μL/min. Sheath flow rate: 30 μL/min, sheath liquid: 100% H_2O. (B) Zoomed in region of (A) from 0.2 - 0.4 min.

Table S1. ivTT reaction conditions.

Reactions / Components	Negative control (μL)	Positive control (μL)	Test ivTT Reaction (μL)
Solution A	80	80	80
Solution B	60	60	60
RNAse inhibitor	4	4	4
PLP (2.5 mM)	8	8	8
Nuclease free water	48	28	36
DNA	0	0	**12 (TA-pD451, 300 ng/μL)**
Transaminase	0	**20 (ATA-117, 1 mg/mL)**	0
Total Volume	200	200	200